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Photoperiodic Responses Controlling the Induction of Adult Diapause and the Determination of Seasonal Form in the Bean Bug, *Riptortus clavatus*

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ABSTRACT—Two photoperiodic responses manifested by adults were compared at 25°C in the bean bug, *Riptortus clavatus* (Thunberg) (Heteroptera, Alydidae). There was on specific instar or stage sensitive to photoperiod that determined both the induction of diapause and seasonal forms. Long-day conditions and constant darkness prevented diapause, whereas short-day conditions induced it. No sex difference was observed in the photoperiodic response curve for the induction of diapause. In males, the curve for the determination of seasonal form was similar to that for the induction of diapause. In females, some long-day adults emerged with body coloration similar to that observed under short-day conditions, but the response curve resembled that in males and that for the induction of diapause in both sexes. Under conditions near critical daylengths, there was a close relationship between diapause and seasonal form, although this relationship was not perfect. These results may indicate a common time-measurement system for the two phenomena.

INTRODUCTION

In many insects, photoperiod controls both morphological polyphenism and diapause [1] and a threshold response is common to the two phenomena [5, 7, 12, 19]. However, Kato and Hasegawa [10] showed that, in *Sasakia charonda* (Lepidoptera, Nymphalidae), the critical daylength for the induction of long-horn larvae is a little longer than that for the induction of larval diapause. In *Aquarius paludum* (Heteroptera, Gerridae), Harada and Numata [8] recently showed that the critical daylength for the induction of macropterous adults is about 45 min longer than that for the induction of adult diapause. It is of interest, therefore, to extend the comparison of the photoperiodic control between diapause and morphological polyphenism in other species.

In the bean bug, *Riptortus clavatus*, Kidokoro [13] was the first to demonstrate that the induction of adult diapause is controlled by photoperiod. Numata and Hidaka [14] examined the photoperiodic response curve for diapause in females. In this species, males also enter an adult diapause, in response to photoperiod [15, 16]. Photoperiod also affects the body coloration of adults, and this can be regarded as an example of seasonal polyphenism [14]. Thus, adults of this species show two photoperiodic responses controlling diapause and seasonal form, respectively. In this study, we compared the photoperiodic response curve between the two responses and determined the stages sensitive to photoperiod. Our results lead us to suggest the existence of a common time-measurement system for the two responses.

MATERIALS AND METHODS

Insects

Adults of *R. clavatus* were collected in legume fields in Kyoto City, Japan (35°00′N, 135°45′E) and their eggs were used for the experiments. Nymphs were reared, as described earlier [14], under various photoperiodic conditions at 25± 1°C. After adult emergence, the insects were

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reared as pairs (one male and one female) in 200-ml plastic cups.

Diapause status

Ten days after adult emergence, the insects were dissected to assess the reproductive status. If yolk deposition was not observed in oocytes, the female was judged to be in diapause [14]. When the width of the erection fluid reservoir, the sac of the ectadene accessory gland, was less than 1 mm, the male was judged to be in diapause [16].

Body coloration

Body coloration was assessed 10 days after adult emergence. Although the coloration of various areas of body surface differed between long-day and short-day adults, the most conspicuous difference was found in the number and area of white spots on the lateral metathorax. In this study, therefore, we calssified adults on the basis of the coloration of the lateral metahorax. Males were classified into five grades and females, which were darker and less variable in body coloration than males, into only three grades (Fig. 1). In the field, males in grades 1–3 and females in grades 1–2 are common in summer, whereas males in grades 4–5 and females in grade 3 are predominant in autumn and in spring before adults of the first generation emerge (Kobayashi and Numata, unpublished). Therefore, we will refer the former as summer forms, and the latter as autumn forms.

Tukey-type multiple comparison test was made for the proportion of diapause and autumn forms [20].

RESULTS

Stage sensitive to photoperiod for the induction of diapause

Insects reared constantly under typical long-day (16L-8D) conditions became nondiapause adults (Fig. 2A). Most females began to lay eggs within 10 days of adult emergence. However, those reared constantly under typical short-day (10L-14D) conditions entered diapause (Fig. 2F). We transferred insects from short-day to long-day conditions and *vice versa* at various stages. When

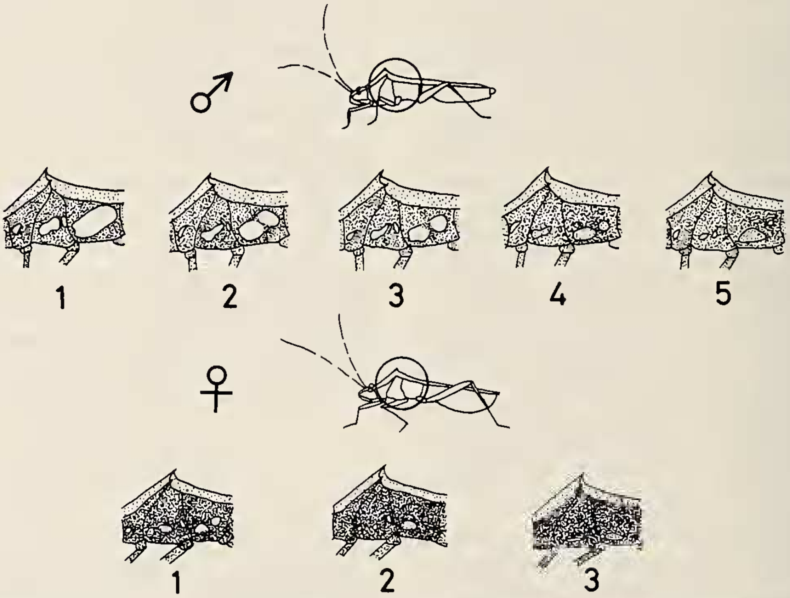

Fig. 1. Grades of body coloration in adults of *Riptortus clavatus,* based on the white spots on the lateral metathorax. Males: grade 1, a single large spot; grade 2, two spots separated by a thin line; grade 3, two spots separated clearly but no black dots in the center of the anterior spot; grade 4, two separate spots and some black dots in the center of the anterior spot; grade 5, the posterior spot is very small. Females: grade 1, two spots with no black dots; grade 2, two spots and some black dots in the anteiror spot; grade 3, no posterior spot.

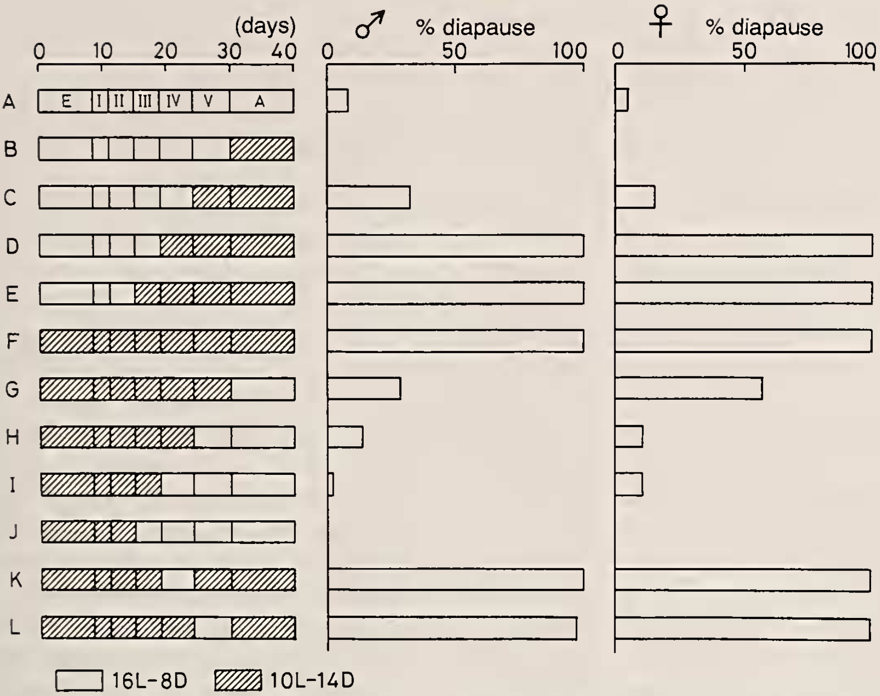

FIG. 2. Stages sensitive to photoperiod for the induction of adult diapause at 25°C in *Riptortus clavatus*. E, egg; I-V, nymphal instars; A, adult stage. $n=19$–52.

transferred from long-day to short-day conditions at adult emergence, the insects failed to enter diapause (Fig. 2B). Exposure to short-day conditions after the fourth nymphal ecdysis showed a significant effect on the induction of diapause ($P<0.001$ in males, $P<0.05$ in females) (Figs. 2A and 2C). All insects entered diapause when transferred from long-day to short-day conditions at the third or the second nymphal ecdysis (Figs. 2D and 2E). When transferred from short-day to long-day conditions at adult emergence, some males sexually matured and some females started vitellogeneisis (Fig. 2G), although no female laid eggs within 10 days of adult emergence. When transferred from short-day to long-day conditions at the fourth, third or second nymphal ecdysis, most or all insects became nondiapause adults and most of the females laid eggs within 10 days of adult emergence (Figs. 2H, 2I and 2J). Thus, the photoperiod during the fourth or the fifth instar appeared to be most important in the induction of diapause. However, exposure to long-day conditions only during the fourth or fifth instar did not prevent diapause (Figs. 2K and 2L). The photoperiod during the first three instars showed a significant effect if insects were exposed to long-day conditions during the fourth instar and transferred to short-day conditions thereafter ($P<0.001$ in both sexes) (Figs. 2C and 2K).

Photoperiodic response curve for the induction of diapause

Figure 3 shows the photoperiodic response curve for the induction of diapause. Short-day conditions from 6L-18D to 13L-11D induced diapause, whereas long-day conditions, from 14L-10D to 24L-0D, prevented diapause. The critical daylength found within the range of natural daylengths, was about 13.5 hr. All adults but one male entered diapause at 13L-11D, whereas no adult did so at 14L-10D. Thus, the change in the incidence of diapause was rather abrupt cross the critical daylength. In a range from 4L-20D to 0L-24D, the incidence of diapause gradually decreased as the photophase decreased, and no adult entered diapause in continuous darkness. Almost

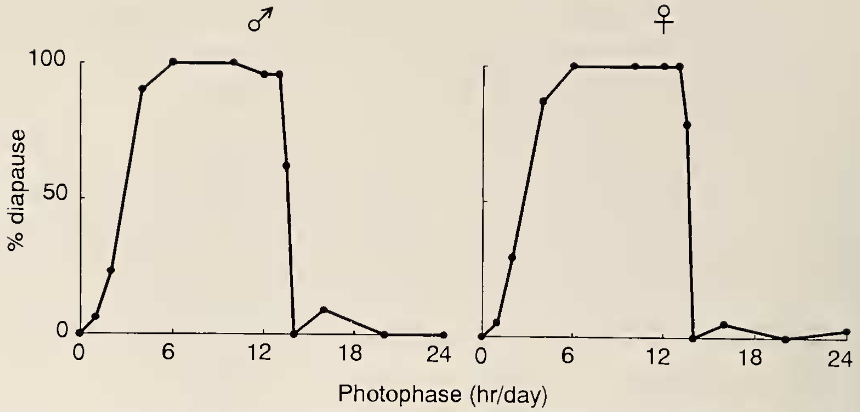

Fig. 3. Photoperiodic response curves for the induction of audlt diapause at 25°C in *Riptortus clavatus*. $n=20$–61

identical photoperiodic response curves were obtained in the two sexes.

Stage sensitive to photoperiod for the determination of seasonal form

We compared body coloration between long-day (16L-8D) and short-day (10L-14D) adults. Under long-day conditions, all male adults belonged to either grade 1, 2 or 3 except for one individual which attained grade 4. Most females were in grade 1 or 2 with about 10% in grade 3 (Fig. 4A). Under short-day conditions, males in grades 4 and 5 were obtained with one individual in grade 3, and all females were in grade 3 (Fig. 4E).

The white spots on the thorax remained conspicuous and their size was not influenced by adult photoperiod. Therefore, the results of the experimental regimes in which only adult conditions differed were combined in Figure 4. Exposure to short-day conditions only during the fifth instar

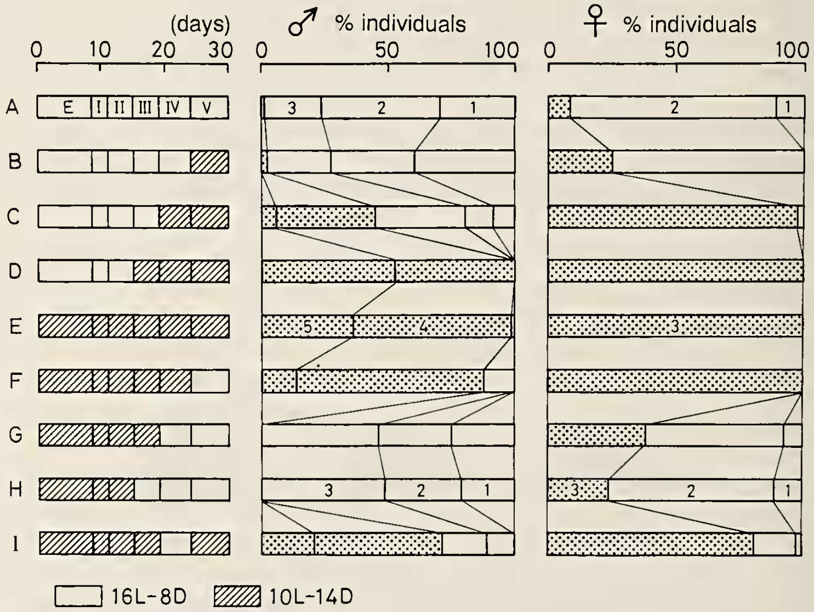

Fig. 4. Stages sensitive to photoperiod for the determination of seasonal form at 25°C in *Riptortus clavatus*. E, egg; I-V, nymphal instars. Numerals on the graph indicate grades of body coloration (see Fig. 1). Shaded areas indicate autumn forms. $n=28$–76.

had no significant effect on the seasonal forms in males ($P>0.05$) but significantly increased the incidence of autumn form in females ($P<0.001$) (Figs. 4A and 4B). When transferred from long-day to short-day conditions at the third nymphal ecdysis, about half the males and most of the females emerged as autumn forms (Fig. 4C). When the transfer was made at the second nymphal ecdysis, all individuals became autumn forms (Fig. 4D). When transferred from sort-day to long-day conditions at the third or the second nymphal ecdysis, all males and more than half of the females became summer forms (Figs. 4G and 4H). When insects were exposed to long-day conditions during the fourth instar and otherwise kept under short-day conditions, some summer forms appeared in both sexes (Fig. 4I). Even though the duration of the fourth instar was shorter than that of the fifth instar, exposure to long-day conditions during the fourth instar produced significantly greater proportion of summer forms than that during the fifth instar ($P<0.05$ in males, $P<0.001$ in females) (Figs. 4F and 4I). Thus, the photoperiod during the fourth instar appeared to be most important in determining the seasonal form. However, the photoperiod before the third nymphal ecdysis showed a significant effect if insects were kept under long-day conditions during the fourth instar and under short-day coditions during the fifth instar ($P<0.001$ in both sexes) (Figs. 4B and 4I). The photoperiod in the fifth instar had also a significant effect in insects exposed to short-day conditions during the first three instars and to long-day conditions during the fourth instar ($P<0.001$ in both sexes) (Figs. 4G and 4I).

Photoperiodic response curve for the determination of seasonal form

Figure 5 shows the photoperiodic response curve for the determination of seasonal form. In males, a range of photoperiod from 6L-18D to 12L-12D produced autumn forms, whereas the from 16L-8D to 24L-0D produced summer forms. The critical daylength falling in the ecologically relevant range was about 13.5 hr, as it was for the induction of diapause. Under extremely short days ranging from 0L-24D to 4L-20D the proportion of autumn forms gradually decreased as the photophase decreased. In continuous darkness, all males became summer forms. The incidence of autumn forms was 81% at 13L-11D at which 96% entered diapause, and 15% under 14L-10D, where all adults failed to enter diapause. Consequently, the change in the incidence of autumn forms near the critical daylength was less abrupt than that in the incidence of diapause (Fig. 6).

All or most females became autumn forms at a photoperiod ranging from 4L-20D to 13.5L-10.5D. Unlike the case for males, some autumn-form females appeared even under long-day conditions (16L-8D to 24L-0D) and in continuous darkness.

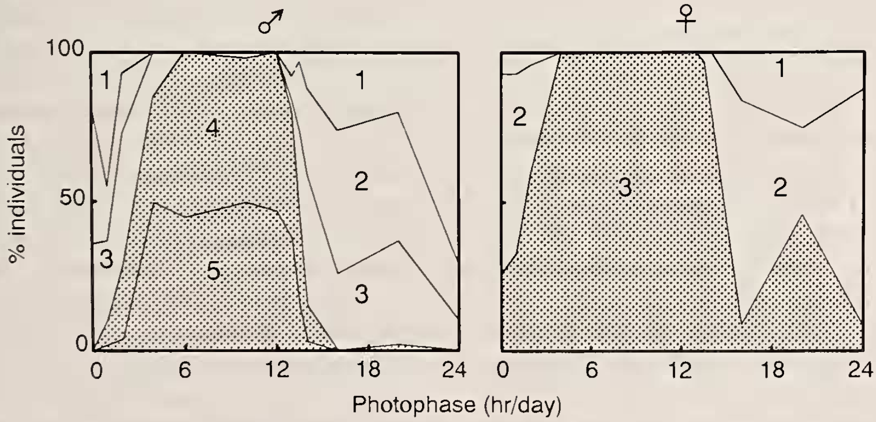

FIG. 5. Photoperiodic response curves for the determination of seasonal form at 25°C in *Riptortus clavatus*. Numerals on the graph indicate grades of body coloration (see Fig. 1). Shaded areas indicate autumn forms. $n=$ 20–61.

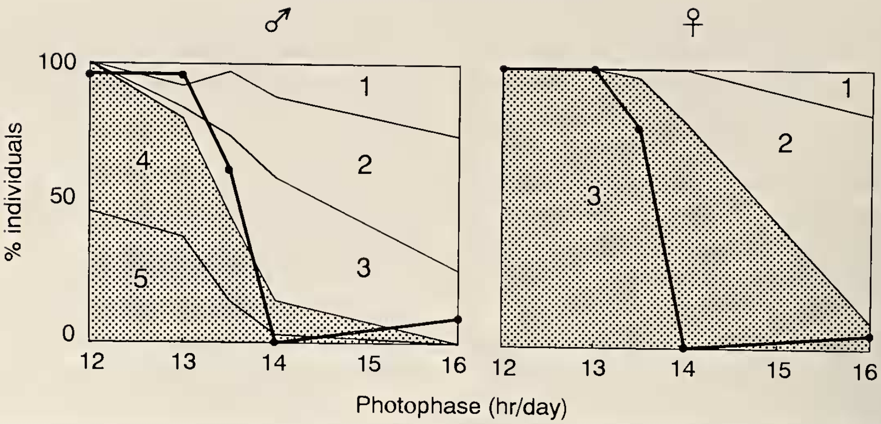

Fig. 6. Comparison of the photoperiodic responses controlling the induction of adult diapause (thick lines) and the determination of seasonal form (thin lines) at 25°C in *Riptortus clavatus*. Numerals on the graph indicate grades of body coloration (see Fig. 1). Shaded areas indicate autumn forms.

Table 1. Relationship between diapause and body coloration in males of *Riptortus clavatus* at 25°C

Photoperiod	No. of individuals						
	Total	Diapause status	Grade of body coloration 1	2	3	4	5
13.5L-10.5D	39	Nondiapause	1	7	6	1	0
		Diapause	0	2	5	11	6
2L-22D	52	Nondiapause	3	11	20	6	0
		Diapause	0	0	3	7	2

The critical daylength falling in the ecologically relevant range slightly longer than 14 hr (Figs. 5 and 6).

Relationship between diapause and seasonal form under conditions near critical daylength

At 13.5L-10.5D and 2L-22D, both diapause and nondiapause adults emerged, and all grades of body coloration were observed. Although the reproductive status and seasonal forms were not perfectly related, there was a significant correlation between them ($P<0.001$ by Fisher's exact probability test for both conditions). Autumn forms tended to enter diapause, and summer forms especially in grades 1 and 2 tended to avert diapause (Table 1).

DISCUSSION

Adults of *R. clavatus* reproduced under long-day conditions from 14L-10D to 24L-0D, whereas they entered diapause under short-day conditions from 6L-18D to 13L-11D at 25°C (Fig. 3). Numata and Hidaka [14] reported previously that this population fails to enter diapause at 4L-20D, although at the other photoperiods tested, their results conform to those of the present study. This discrepancy observed at the extremely short daylengths may reflect the absence of any selective pressure because such conditions are normally not encountered in this population in nature.

There is no specific instar or stage that is sensitive to photoperiod which determines the induction of both diapause and seasonal form in *R. clavatus* (Figs. 2 and 4). In *Orgyia thellina* (Lepidoptera, Lymantriidae), there is no difference in terms of the stage sensitive to photoperiod between the induction of egg diapause and the determination of wing form [12]. In *Polygonia c-aureum* (Lepidoptera, Nymphalidae), the stage sensitive to photoperiod for the induction of adult diapause extends a little longer than that for the determination of seasonal form [7]. With respect to diapause, *R. clavatus* remains sensitive to photoperiod even after adult emergence; a change in photoperiod in the adult stage can terminate or induce diapause [14]. The seasonal form is, of course, determined before adult emergence and, therefore, there is a difference between the two responses in the stage to which the photoperiodic influence extends (Figs. 2 and 4).

In male *R. clavatus,* the seasonal form and diapause are controlled by photoperiod in similar manners (Figs. 3 and 5). The range of photoperiods inducing the autumn form in males (3L-21D to 13.5L-10.5D) was narrower than that in females (2L-22D to 14L-10D). Thus, a small difference appears to exist between the sexes in terms of the photoperiodic control for the determination of seasonal form in *R. clavatus.* Sex differences in the photoperiodic control for the determination of seasonal form have been reported in other species [11, 12, 17]. In *R. clavatus,* however, the photoperiodic response curves for induction of the autumn form would become nearly identical in the two sexes if we include grade 3 males as autumn forms (Fig. 5). Thus, the apparent sex difference in the photoperiodic response curves should be due to an artifact because the seasonal form was divided into 5 grades in males and only 3 in females. Therefore, it seems likely that both sexes share the same time-measurement system for the determination of seasonal form.

The reproductive status in adults of *R. clavatus* can be one of two possible conditions, namely, diapause or nondiapause, as it is in other many insects [1]. This species has two distinct thresholds in the photoperiodic response curve for the induction of diapause (Fig. 3). This is also true for the determination of seasonal form, although the variation in body coloration is continuous (Fig. 5). Both phenomena are controlled by the same critical daylengths at least in males (Figs. 3 and 5). Furthermore, at a photoperiod near the critical level, a significant correlation is observed between diapause and seasonal form (Table 1). We propose, therefore, that a common time-measurement system is shared by the controls of seasonal form and diapause.

In the range close to the critical daylength, the response for the determination of seasonal form is somewhat quantitatively related to the duration of the photophase (Fig. 6). In *O. thellina,* the photoperiodic response curve for pupal melanism resembles the curves for the determination of diapause and wing form which are controlled by all-or-none response. However, the degree of melanization is quantitatively related to the duration of the photophase near the critical daylengths [12]. Spieth and Sauer [18] suggested that the quantitative measurement of photoperiod is involved in an apparently threshold response in *Pieris brassicae* (Lepidoptera, Pieridae). The mechanisms controlling threshold and graded responses deserve further study.

In *O. thellina,* macropterous adults lay nondiapause eggs whereas brachypterous ones lay diapause eggs, and these two photoperiodically induced variations are rarely separated from one another [12]. Kimura and Masaki [12] suggested that the induction of diapause and the determination of wing form are coupled to a common mechanism of photoperiodic response. A close correlation is also found between diapause and seasonal form, in the adult stage of *P. c-aureum* [7, 9]. In this case, a neuroendocrine factor from the pars intercerebralis produces the summer form [4, 6], and a hormone from the corpora allata stimulates ovarian maturation [2]. Moreover, another neuroendocrine factor from the pars intercerebralis activates the corpora allata [3]. Endo *et al.* [7] suggested that *P. c-aureum* uses a common clock mechanism to control the two kinds of seasonal diphenism even though the neuroendocrine factors responsible for them are different.

In *R. clavatus,* the correlation between seasonal form and diapause is not as close as it is in *O. thellina* and *P. c-aureum* (Table 1, Fig. 6). In *R. clavatus,* therefore, different effector systems seem to be involved in the two responses, and the connection between the two mechanisms may be less tight than it is in the other two species mentioned. Adult diapause in *R. clavatus* is due to the inactive secretion of juvenile hormone from the corpora allata [15, 16], but the endocrine mechanism controlling the seasonal form remains to be defined.

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